DEVELOPMENT OF INTRAVAGINAL LIPOSOMAL GEL OF CLOMIPHENE CITRATE FOR TREATMENT OF POLYCYSTIC OVARY SYNDROME

SYNOPSIS OF THE THESIS

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DECLARATION

I hereby declare that the dissertation synopsis entitled "**Development of Intravaginal Liposomal gel of Clomiphene Citrate for treatment of Polycystic Ovary Syndrome**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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1) INTRODUCTION

1.1) Polycystic Ovary Syndrome in the Indian Subcontinent

Polycystic ovary syndrome (PCOS) may clinically be manifested in young women of reproductive age as oligoovulation, biochemical or clinical hyperandrogenism (hirsutism, male pattern balding, acne, acanthosis nigricans), and polycystic ovaries,[1] but PCOS has a long prodrome with detectable abnormalities that present as the metabolic syndrome (hyperinsulinemia, obesity, dyslipidemia [decreased high-density lipoprotein cholesterol and hypertriglyceridemia], hypertension, atherosclerosis, increased risk of development of type 2 diabetes and cardiovascular disease) throughout the life of affected women. There is considerable heterogeneity in the signs and symptoms among women with PCOS, and these may change over time for an individual. [2,3]

PCOS may be diagnosed by the presence of two of three major recommended criteria: (1) oligomenorrhea, (2) any form of hyperandrogenemia, either clinical (hirsutism and acne), or endocrine (the hormonal diagnosis of high androgen levels), and (3) the ultrasound picture of polycystic ovaries, following the exclusion of related disorders, such as Cushing's syndrome, hyperprolactinemia, thyroid dysfunction, androgen-producing tumors, and nonclassic adrenal hyperplasia.[4] Normal ovulatory women with polycystic ovaries (PCO), who exhibit only the ovarian morphology, are not considered to have PCOS. Among different criteria, hyperandrogenemia, specifically elevated bioavailable testosterone, and the recognition of oligo-ovulation are academically conducive in the diagnosis. The presence of either 12 or more follicles, measuring 2 to 9 mm in diameter, or an increased ovarian volume (>10 cm^3) are criteria required to define PCO. Polycystic ovaries have been reported in 80 to 100% of women with PCOS. Overall, the ultrasonography (USG) findings are not sufficient for the diagnosis of PCOS as the typical USG findings of PCO are demonstrated in 20 to 25% of regularly ovulating healthy women. The presence of a single polycystic ovary is sufficient to provide the diagnosis.[5] Estimates of the prevalence of polycystic ovaries in migrant Asian Indians hover around 52%.[6]

PCOS has been found to be more prevalent in South Asian women residing in the United Kingdom than in Caucasians (white-skinned; of European origin.).[8] Significant associations between PCO and menstrual irregularity, infertility, the Ferriman and Gallwey score for body hair distribution, the presence of acanthosisnigricans,(dark, velvety patches) and the fasting blood glucose concentration have been reported among Asian Indians.[6]

Menstrual irregularity is the most common gynecologic presentation of PCOS. Oligomenorrhea has been observed in 85 to 90% of women with PCOS, and as many as 30 to 40% of amenorrheic patients have PCOS.[9] The prevalence of infertility, caused mainly by anovulation in PCOS women, varies between 35% and 94%.[10]

Hyperandrogenism is the key endocrine abnormality of PCOS, the prevalence of hirsutism in PCOS women ranging from 17 to 83%. It is recognized that hirsutism is in part ethnically determined, being more common in women with dark skin.[11] Hirsutism is as common a problem in the Kashmir Valley (India) as elsewhere in the world. In a prospective, randomized study to assess the prevalence of hirsutism and study its etiology in the Kashmir Valley of the Indian subcontinent. Insulin resistance (IR) is central to the pathogenesis of PCOS8 and has been associated with endothelial dysfunction, which is considered the initial step in the process of atherosclerosis.[13] the association between increased IR and PCOS is a consistent finding in all ethnic groups.[14]

Epidemiologic studies confirm a high prevalence of type 2 diabetes[18] that has now reached epidemic levels in Asia.[19] Insulin resistance appears to be the most relevant feature of the metabolic syndrome and is often the precursor of diabetes mellitus.[13] A significantly higher prevalence of obesity, central obesity, hypertension, high triglycerides, and low high-density lipoprotein (HDL) ($p<0.01$) has been reported in Asian Indians with insulin resistance syndrome (IRS).[20]

1.1.1) Pathogenesis

A complete understanding of the underlying pathophysiology of PCOS is still lacking. Because of the heterogeneity of this disorder, there are most likely multiple underlying pathophysiologic mechanisms. Several theories have been proposed to explain the pathogenesis of PCOS. 1) An alteration in gonadotropin-releasing hormone secretion results in increased luteinizing hormone (LH) secretion. 2) An alteration in insulin secretion and insulin action results in hyperinsulinemia and insulin resistance. 3) A defect in androgen synthesis that results in increased ovarian androgen production. [21]

1.1.2) Treatment

Treatment goals should include maintaining a normal endometrium, antagonizing the actions of androgens on target tissues, reducing insulin resistance (when present), and correcting anovulation (ovaries do not release an oocyte during a menstrual cycle). The selection of therapy for PCOS generally depends on the physical symptoms and patients' desire for childbearing and may be broadly categorized into therapy for

(i) Symptomatic control

(ii) Fertility management where conception is the priority

(iii) Long-term complications of PCOS.

For obese women with PCOS, weight loss should be considered as a first option.

A) Symptomatic Control includes: oral contraceptive pills (OCPs), Insulin sensitizing drugs, Drug used in treatment of Hirsutism.

B) Fertility Management

Ovulation induction: Several nonpharmacologic (exercise, diet modifications, and weight loss) and pharmacologic (clomiphene citrate, aromatase inhibitors, insulin sensitizers [metformin, rosiglitazone, pioglitazone], gonadotropins) approaches, used alone or in combination, may be used to achieve ovulation.

Insulin Sensitizing Agents: The safety profile of Pioglitazone and rosiglitazone remains to be established, hence, metformin must be used as the first-choice insulin sensitizing drug.

C) Management of Long-term Complications: Lifestyle interventions, Management of the metabolic syndrome.[22]

1.1.3) Liposomal gel

A liposome is a tiny vesicle consisting of an aqueous core entrapped within one or more natural phospholipids forming closed bilayered structures. Liposomes have been extensively used as potential delivery systems for a variety of compounds primarily due to their high degree of biocompatibility and the enormous diversity of structures and compositions. The lipid components of liposomes are predominantly phosphatidylcholine derived from egg or soybean lecithins. Liposomes are biphasic a feature that renders them the ability to act as carriers for both lipophilic and hydrophilic drugs. It has been observed that drug molecules are located differently in the liposomal environment and depending upon their solubility and partitioning characteristics, they exhibit different entrapment and release properties. Lipophilic drugs are generally entrapped almost completely in the lipid bilayers of liposomes

and since they are poorly water soluble, problems like loss of an entrapped drug on storage are rarely encountered. Hydrophilic drugs may either be entrapped inside the aqueous cores of liposomes or be located in the external water phase. Noteworthy is that the encapsulation percentage of hydrophilic drugs by liposomes depends on the bilayer composition and preparation procedure of the liposomes.[23-24]

2) Review of Literature

- Refai et al, (2017) prepared polymer-coated liposomes for vaginal delivery of sildenafil citrate. Liposomal formulation was prepared by thin-film method using different phospholipid:cholesterol ratios. On evaluation The in vitro release studies showed prolonged release of sildenafil from coated liposomes as compared to uncoated liposomes and sildenafil solution. Ex vivo permeation study revealed the enhanced permeation of coated relative to uncoated liposomes.[25]

- Saini et al, (2016) developed Niosomal thermosensitive gel containing metformin for the treatment of polycystic ovary syndrome through intraveginal route. They found that intravaginal MTF-HCl-SUNs-Gel and intravaginal MTF-HCl-C-SUNs-gel exhibited remarkable alterations, recruitment and development of normal follicles in addition tonormalization of level of various hormones in PCOS as compare to oral MTF-HCl solution.[26]

- Manne et al, (2013) prepared lyophilized liposomal gel of an antiviral drug for intravaginal delivery. In Evalution of liposomal gel they demonstrated that The redipsersion of freeze dried forms in SVF was found to be slow and its ex-vivo retention time was found to be 12 hrs while acyclovir gel retained only for 8.25 hrs. The tablet and gel released 96.93±0.15% acyclovir within 6 hrs and 92.31±0.31% by 8 hrs respectively while freeze dried forms could sustain the release upto 12 hrs. From the stability studies the optimum storage condition was found to be 4-8°C.[27]

- Gupta et al, (2012) developed liposome gel based containing protein CN54gp140. They evaluated CN54gp140 liposomes for mean vesicle diameter, polydispersity, morphology, zeta potential and antigen encapsulation efficiency before being incorporated into hydroxyethyl cellulose (HEC) aqueous gel and subsequently lyophilised to produce a rod-shaped solid dosage form for practical vaginal application. The lyophilised liposome–HEC rods were evaluated for moisture content and redispersibility in simulated vaginal fluid. Since these rods are designed to revert to gel form following intravaginal application, mucoadhesive, mechanical (compressibility and hardness) and rheological properties of the reformed gels were evaluated. The liposomes exhibited good encapsulation efficiency and the gels demonstrated suitable mucoadhesive strength. The freeze-dried liposome–HEC formulations

represent a novel formulation strategy that could offer potential as stable and practical dosage form.[28]

- Allahbadia et al (2008), demonstrated prevalence of POCD in Indian subcontinent. They elaborated that Polycystic ovary syndrome (PCOS) is a complex, multifaceted, heterogeneous disorder that affects 5 to 10% of women of reproductive age and summarized that Irrespective of geographic locations, a rapidly increasing prevalence of polycystic ovarian insulin resistance syndrome, excess body fat, adverse body fat patterning, hypertriglyceridemia, and obesity-related disease, such as diabetes and cardiovascular disease, have been reported in Asian Indians, suggesting that primary prevention strategies should be initiated early in this ethnic group. In lieu of the epidemic increase in the prevalence of obesity and diabetes mellitus in most industrialized countries including China and India owing to Westernization, urbanization, and mechanization, and evidence suggesting a pathogenetic role of obesity in the development of PCOS and related infertility, active intervention to combat the malice of these disorders is warranted.[29]

- Pavelic et al (2001) prepared Liposomal gels for vaginal drug delivery to provide sustained and controlled release of appropriate drug for local vaginal therapy. Liposome was prepared using five different method. Two liposomal preparations (proliposomes and polyol dilution liposomes) were optimized and tested for their in vitro stability in media that simulate human vaginal conditions (buffer, pH 4.5). In vitro release studies of liposomes incorporated in these gels (Carbopol 974P NF or Carbopol 980 NF) confirmed their applicability as a novel drug carrier system in vaginal delivery.[30]

- Kousta et al (1997) demonstrated us of clomiphene citrate in induction of ovulation. Clomiphene citrate has been used for induction of ovulation since 1962. It is the treatment of first choice in women with ovulatory disorders who are normally oestrogenized, i.e. predominantly those with polycystic ovaries (PCO). Clomiphene citrate has both oestrogenic and anti-oestrogenic properties. The majority of women with 'pure' anovulatory infertility respond to treatment with clomiphene citrate.[31]

- Jain et al, (1996) developed Liposome Based Contraceptive System for Intravaginal Administration of Progesterone. They found that liposomes was encapsulate nearly 98% of the drug. The in vitro drug diffusion across she-goat vaginal membrane from the liposomal formulation was found to follow near zero order kinetics.[32]

- Buckler et al (1992) demonstrated effect of progesterone in PCOS by veginal route of administration. They found that vaginal progesterone administration, in a dose of 100 mg twice daily, produced circulating progesterone concentrations within the mid-luteal phase range, and this dose exerted only a negative feedback effect on LH secretion. Finally, physiological serum progesterone levels suppressed the endogenously raised serum LH level in PCOS to normal.[33]

3) Rational & Objective

The polycystic ovary syndrome (PCOS) is a multifaceted disease characterized by clinical or biochemical androgen excess and/or chronic anovulation and/or polycystic ovaries on ultrasonography. PCOS is the most common cause of anovulatory infertility, affecting approximately 5–10% of women of reproductive age. Furthermore, PCOS is associated with low level of follicle stimulating hormone (FSH) and high level of luteinizing hormone (LH). In parallel, high level of LH triggers the secretion of estrogen, testosterone and dihydroepiandrosterone sulphate (DHES). This ultimately leads to development of cyst in the ovary.[27] Administration of clomiphene citrate (CC) is the standard treatment for PCOS patients with anovulatory infertility. Clomiphene citrate (CC) is still holding its place as the first-line therapy for ovulation induction in these patients. CC contains an unequal mixture of two isomers as their citrate salts, enclomiphene and zuclomiphene. Zuclomiphene is much the more potent of the two for induction of ovulation, accounts for 38% of the total drug content of one tablet and has a much longer half-life than enclomiphene, being detectable in plasma following its administration. CC is capable of inducing a discharge of FSH from the anterior pituitary and this is often enough to reset the cycle of events leading to ovulation into motion. This is achieved indirectly, through the action of CC, a non-steroidal compound closely resembling an estrogen, in blocking hypothalamic estrogen receptors, signalling a lack of circulating estrogen to the hypothalamus and inducing a change in the pattern of pulsatile release of GnRH. Standard practice is to administer CC for 5 days from the second or third day of the menstrual cycle, starting with 50mg/day and increasing to 250mg/day. However managed care studies have shown that the most effective dosage is 100–150mg/day and over 75% of ovulations occur within these dosages. However, despite favorable properties, oral administration of clomiphene citrate is associated with low water solubility, side effects, in PCOS that ultimately influence the patient compliance.

So far, as no effective single drug is identified for PCOS treatment, the treatment of PCOS is a challenging problem worldwide at present. Due to its complex pathogenesis and unrecognized etiology, there is no effective preventive measure available for PCOS until now. Inspite of the tremendous progress made in the development of drug delivery system, there is an urgent need to find out alternate therapeutic regimen to control PCOS syndrome

Intra-Veginal route offers large surface area, rich blood supply, avoidance of first-pass metabolism, high permeability and greater retention of drugs. Hence, several delivery systems have been investigated for the delivery of therapeutic moieties through vaginal route.

Furthermore, colloidal vesicular systems like liposomes have also been amalgamated with mucoadhesive drug delivery systems to enhance the permeation rate of drugs. Thermosensitive gels undergo sol-gel transition after local administration in physiological conditions which is a bench-mark in drug delivery applications.[34-38.26]

Objective:

- To carryout Preformulation study.
- Compatibility study between drug and excipients.
- To formulate liposomal gel for PCOS, through vaginal route.
- To optimize the ratio of ingredients for successful formulation of gel (optimization).
- To evaluate the optimized formulation for various parameter (IN-Vitro study)
- To check the stability of optimized formulation.
- To evaluate the optimized formulation for biological activity (IN-Vivo study)
-

Beside all these objectives our aim is

- To enhance drug loading
- To enhance permeation of drug
- To reduce side effect of drug
- Improve patient compliance and therapeutic efficacy

4) Plan of Work

- Literature Survey
- Preformulation study of Clomiphene citrate
- Preformulation study of Clomiphene citrate
    - Melting point
    - FT-IR, DSC
    - Determination of absorption maxima using uv spectroscopy
    - UV spectroscopy method development and Validation
    - HPLC method development
    - Solubility study
    - XRD
- Compatibility study between drug and excipients using FT-IR
- Development of liposome of Clomiphene citrate
    - Preparation of liposome using different lipids, and other ingredients and many parameters.
    - Optimization using design of experiment software
- Evaluation of Liposome
    - pH
    - FT-IR
    - XRD
    - DSC
    - Globule size
    - Drug loading
    - Drug entrapment
    - Transmission electron microscopy (TEM)
- Development of liposomal gel of optimized formulation
- Evaluation of liposomal gel formulation
    - pH
    - Drug content
    - Drug release study
    - Viscosity
    - Spreadibility

- Texture
- In-vitro Percentage Cumulative Drug release (using Dialysis membrane)
- Drug release kinetic

➢ Stability study of optimized formulation

➢ In-vivo Study

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